Chapter

New Approaches to the Treatment of Chronic Myeloid Leukemia

Lyudmyla P. Shvachko, Svitlana Antonenko, Michael Zavelevich, Irene Gartovska, Mykhailo Dybkov, Dmytro Gur`yanov and Gennadii Telegeev

Abstract

While the specific inhibitors of BCR-ABL tyrosine kinase activity have gained well-deserved recognition in the frontline therapy of chronic myeloid leukemia (CML), acquired therapy resistance represents a serious problem, preventing long-term remission in CML patients. Moreover, in some patients, the resistance mechanisms are BCR-ABL-independent. Therefore, the search for alternative and complementary therapies is currently in the spotlight. The differentiation therapy may represent one of the possible approaches. Alpha-tocopherol may be one of the agents possessing differentiation potential in CML blast cells. We demonstrated that alpha-tocopherol and G-CSF upregulate the expression of several genes associated with the differentiation of K562 CML cells in vitro, in particular, the gene coding for the myeloid master regulator transcription factor C/EBPα. At the same time, a significant down-regulation of the expression of genes associated with the stemness phenotype, such as *SNAI1, ALPP*, and *POU5F1* was observed. Other possible targets may be searched among the enzymes preventing the degradation of the BCR-ABL protein. The data on the specific inhibition of deubiquitinase USP1 activity in vitro in K562 cells have been presented. A vast array of other targetable enzymes interacting with the BCR-ABL protein and affecting its stability and activity remains an extensive field for further study. A putative role of protein phosphatase 2A in this context is discussed.

Keywords: chronic myeloid therapy, BCR-ABL, differentiation therapy, stemness phenotype, USP1 deubiquitinase, K562 cells

1. Introduction

Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell (HSC) disorder characterized by a reciprocal translocation (t(9;22)(q34;q11)) resulting in a *BCR-ABL1* fusion gene and the chimeric p210 BCR-ABL1 protein possessing constitutive kinase activity [1, 2]. The specific inhibitors of tyrosine kinase activity (TKI), such as imatinib, have found deserved recognition in the frontline therapy of CML [3]. Nevertheless, acquired therapy resistance represents a serious problem,

preventing long-term remission in CML patients despite advances in the development of more potent TKIs [4]. Meanwhile, various mechanisms are involved in the development of resistance to therapy that are not completely dependent on BCR-ABL1 activity [5]. Therefore, the search for alternative therapies, including differentiation therapy, is currently in the limelight. Over the past years, the inadequacy of many CML therapies has been shown to result from their failure to target LSCs. The control over the differentiation of CML LSCs may represent a useful therapeutic approach [6]. In fact, various agents, including growth factors, cytokines, chemical substances, etc., have been identified as differentiating agents for CML cells in vitro. Nevertheless, the mechanisms mediating the differentiation effects may be diverse, affecting different regulatory pathways in leukemic cells. The analysis of the signaling pathways and their components as targets of the induced differentiation of CML cells is an important step in developing novel therapeutic strategies in the context of cell differentiation therapy. Here, we present our recent data on the expression of the genes involved in the differentiation of CML cells and the genes coding for stemness biomarkers in K562 cells exposed to alpha-tocopherol as a putative agent capable of contributing to CML cell differentiation.

Actually, several alternative and complementary approaches for CML treatments are currently underway in the hope of overcoming resistance to TKI, as well as identifying different pathways and molecular targets that prevent uncontrolled proliferation of leukemic blast cells in CML. Such strategies are directed not at the inhibition of the constitutively activated tyrosine kinase but rather at the degradation of this chimeric protein and the eventual elimination of cells from the pathological clone [7]. Although various modalities of BCR-ABL1 degradation are being developed and tested in in vitro systems, the lack of sufficient specificity is still a problem to be solved. Targeting deubiquitinating enzymes may provide an alternative strategy for the selective modulation of BCR-ABL1 protein levels in CML cells, which has proven to be effective in in vitro studies [8]. The data on the use of specific inhibitors of the deubiquitinase USP1 activity in vitro in K562 cells will be discussed.

The future prospects of the research should be based on the analysis of the proteins interacting with BCR-ABL in hematopoietic stem cells and leukemic blasts [9]. Some challenging novel strategies, such as the specific reactivation of protein phosphatase 2A (PP2A), have been proposed [10]. The recent literature data on the interplay between the activity of BCR-ABL1 and PP2A in CML cells and the putative ways to modulate PP2A activity will be disclosed in the context of further studies of PP2A-reactivating modalities. Moreover, the recent literature data on the interplay between the activity of BCR-ABL1 and PP2A in CML cells and the putative ways to modulate PP2A activity suggest that alpha-tocopherol is among the known PP2A-reactivating molecules.

2. Alpha-tocopherol and G-CSF change the expression of genes associated with the differentiation of K562 CML cells while downregulating EMT-associated stemness biomarkers

Myeloid master regulator transcription factor CCAAT/enhancer-binding protein alpha (C/EBPα) is considered as a major molecular trigger of myelopoiesis, however, CML blast cells lose their differentiation potential and acquire uncontrolled

proliferation [11]. Down-regulation of C/EBPα is correlated with the progression of CML to blast crisis, while the restoration of C/EBPα expression in the BCR-ABL + cell line by TKI results in terminal granulocytic differentiation [12]. These findings, along with many others, suggest that C/EBPα could be considered a putative target in differentiation therapies for myeloid leukemias, including CML.

Historically, a variety of chemical agents were found to be able to induce differentiation of CML cells in vitro, many of them being rather toxic and not intended for any practical purposes [13]. Meanwhile, it has long been known that some of the hematopoietic leukemia cell lines of myeloid origin, such as K562 or CML blasts, may be induced to differentiate by various cytokines [14].

In our previous chapter in the IntechOpen monograph [15], we presented data on the putative role of alpha-tocopherol in the targeted induction of the myeloid master regulator transcription factor C/EBPα in K562 cells. Earlier, we also demonstrated the ability of alpha-tocopherol to restore C/EBPα expression, resulting in C/EBPα-mediated expression of the gene for the G-CSF receptor (*CSF3R*) in K562 cells [16]. Meanwhile, G-CSF-R is expressed not only on myeloid cells such as neutrophils but also on HSCs and progenitor cells [17]. Taken together, these facts suggest a functional interrelationship between C/EBPα, G-CSF-R, and G-CSF [18, 19].

The possible involvement of G-CSF in the differentiation of CML blast cells has not yet been studied in detail. The loss of G-CSF and G-CSFR is a characteristic feature associated with the arrested differentiation of CML blast cells. G-CSF is currently used in CML patients, improving responsiveness to TKI therapy. It is known that normal myelopoiesis in bone marrow is supported by G-CSF, which is constitutively produced by bone-forming osteoblasts [20–22]. Meanwhile, tissue non-specific (liver/bone/kidney) alkaline phosphatase (TNAP), representing an osteogenic differentiation regulator of osteoblasts, seems to be important in supporting the HSC niche in bone marrow [23]. G-CSF and TNAP can be suggested as mutually dependent markers of HSC differentiation. Moreover, TNAP, encoded by the *ALPL* gene, is detectable in differentiated neutrophils and monocytes and is induced by G-CSF treatment [24].

Previously, we have shown that in K562 CML cells, the *ALPL* gene was not expressed, while the expression of the aberrant isoform of alkaline phosphatase, namely leukemic stem cell-associated placental-like alkaline phosphatase (PLAP), was observed [15, 25]. This aberrant isoform of alkaline phosphatase is considered potentially useful as a tumor-associated marker [26]. Moreover, according to our data, alpha-tocopherol is effective in restoring the expression of C/EBPα and TNAP in K562 cells, both being tightly associated with the possible reactivation of myeloid differentiation potential [25].

In the present study, we further explored the differentiation potential of alpha-tocopherol and G-CSF by analyzing the expression of *CEBPB* and *ALPL* genes, which encode factors critical for myeloid differentiation, versus the expression of genes encoding leukemic stemness-associated transcription factors SNAIL and OCT4 in K562 CML cells.

K562 cells were treated for 72 hours either with alpha-tocopherol acetate at a dose of 100 μM (300 mg/mL stock solution; Lekchim, Ukraine) or G-CSF (Filstim, Biofarma, Ukraine) at a dose of 1 μg/mL (10,000 IU). A real-time RT-PCR was performed using the following primers (**Table 1**). The gene expression was quantified using the 2Δ-Ct method with normalization to *GAPDH* mRNA expression.

Gene	Forward primer	Reverse primer
ALPL	5′-TGGCCGGAAATACATGTACCC-3′	5′-TTCCGTGCGGTTCCAGATG-3′
ALPP	5′-AAGGGCAGAAGAAGGACAAA	5′-GTCGTGTTGCACTGGTTAAAG
CEBPB	5′-CAA GAA CAG CAA CGA GTA CCG-3′	5′-GTC ACT GGT CAA CTC CAG CAC-3′
CDH1	5′-TGCCCAGAAAATGAAAAAGG-3′	5′-GTGTATGTGGCAATGCGTTC-3′
SNAI1	5′-CAGACCCACTCAGATGTCAA-3′	5′-CATAGTTAGTCACACCTCGT-3′
POU5F1	5′-GCACTGTACTCCTCGGTCCCTTTCCC-3′	5′-CTTCCCTCCAACCAGTTGCCCCAAAC-3′
GAPDH	5′-CGCTCTCTGCTCCTCCTGTT-3′	5′-CCATGGTGTCTGAGCGATGT-3′

Table 1.
Primers used in the study.

Our findings demonstrate that either alpha-tocopherol or G-CSF upregulated *CEBPB* expression, but alpha-tocopherol caused 2.54 times higher upregulation of the gene compared to G-CSF (**Figure 1A**). Both single agents also upregulated *ALPL* expression to the same extent (**Figure 1B**). Coincidentally, with the expression of factors affecting differentiation of myeloid cells, we observed a significant increase in the expression of the *CDH1* gene (E-cadherin) (**Figure 1C**).

Although E-cadherin is not associated directly with the differentiation of myeloid cells, the treatment of K562 cells with a differentiating agent such as phorbol ether results in an increasing expression of E-cadherin [27]. Such upregulation of the *CDH1* gene, coinciding with the increasing expression of markers of myeloid differentiation such as C/EBP and TNAP induced by alpha-tocopherol or G-CSF, prompted us to analyze the expression of several transcription factors that are usually upregulated in the setting of the loss of E-cadherin. It is known that the activation of transcription factors such as SNAIL, SLUG, TWIST1, ZEB1, ZEB2, and several others, which bind to E-box sequences in the *CDH1* gene promoter, negatively regulates E-cadherin expression [28]. Moreover, these factors have been found to be associated with the maintenance of cell pluripotency and stemness in hematological malignancies [29].

While EMT has been implicated in promoting the stemness of cancer cells, which is important for tumorigenesis and metastasis in solid tumors, the role of EMT and the major transcription factors involved in this process in the case of hematopoietic malignancies remains to be elucidated. Several studies have assumed a role for such EMT-related factors as SNAIL, TWIST, and others in the pathogenesis of myeloid malignancies [30, 31].

We found a significant down-regulation of *SNAI1, ALPP*, and *POU5F1* expression in K562 cells incubated with alpha-tocopherol or G-CSF. Alpha-tocopherol was as effective as G-CSF in decreasing *ALPP* and *POU5F1* RNA expression, while its effect on *SNAI1* expression was less noticeable compared to G-CSF (**Figure 2**).

Earlier, we have already found PLAP expression in peripheral blood cells of patients with CML in blast crisis, acute myeloid leukemia, and polycythemia vera, as well as in K562 cells [25]. In this study, we showed that PLAP downregulation is triggered not only by alpha-tocopherol but also by G-CSF as a specific inducer of myeloid differentiation. Moreover, the changes in the expression of PLAP caused by the differentiation agents are paralleled by the downregulation of the transcription factors SNAIL and OCT4 which are associated with the maintenance of CSC stemness.

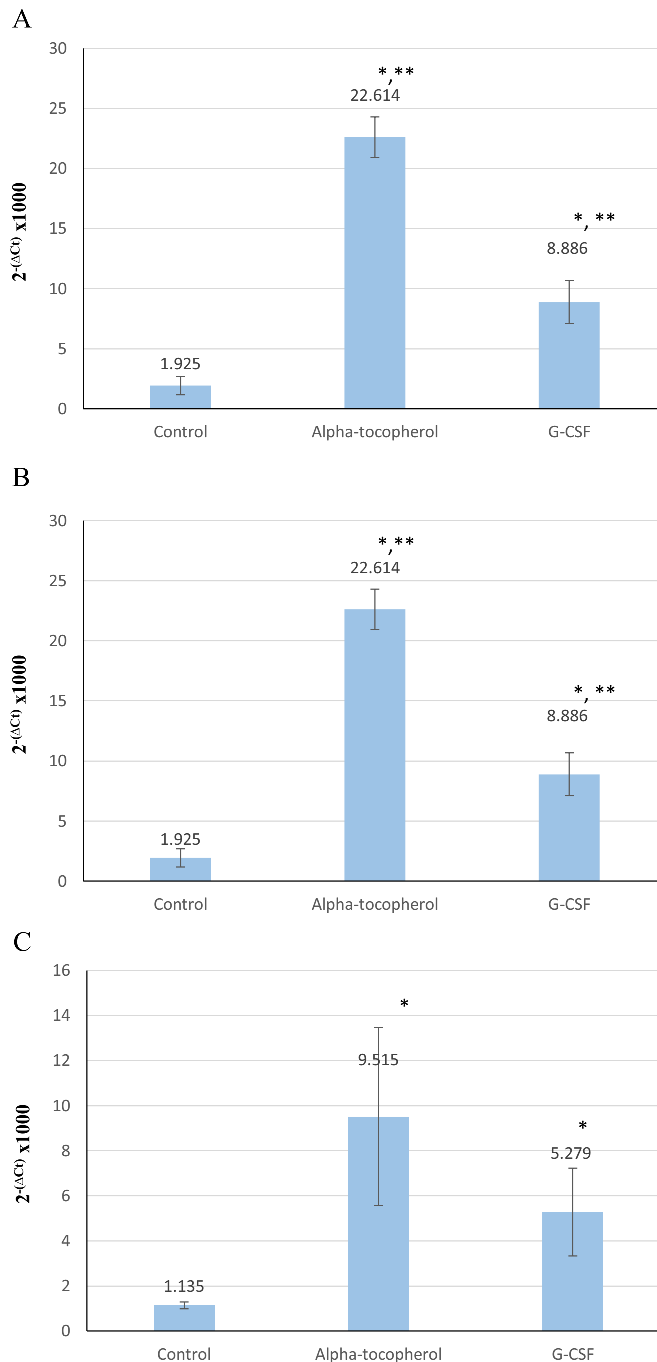

Figure 1.
The relative mRNA expression levels of the CEBPB *(A),* ALPL *(B), and* CDH1 *(C) genes. K562 cells were incubated either with alpha-tocopherol acetate at a dose of 100 μM (300 mg/mL stock solution; Lekchim, Ukraine) or G-CSF (Filstim, Biofarma, Ukraine) at a dose of 1 μg/mL (10,000 IU) for 72 h. Total RNA was extracted and converted to cDNA using Qiagen's QuantiTect Rev. Transcription Kit (Qiagen, Germany). A real-time RT-PCR was performed using the SYBR Green protocol. All the experiments were conducted in triplicate. The gene expression was quantified using the* $2^{-\Delta Ct}$ *method with normalization to GAPDH mRNA expression.* * p *< 0.01 compared to control cells;* ** p *< 0.01 between the effects of alpha-tocopherol and G-CSF treatments.*

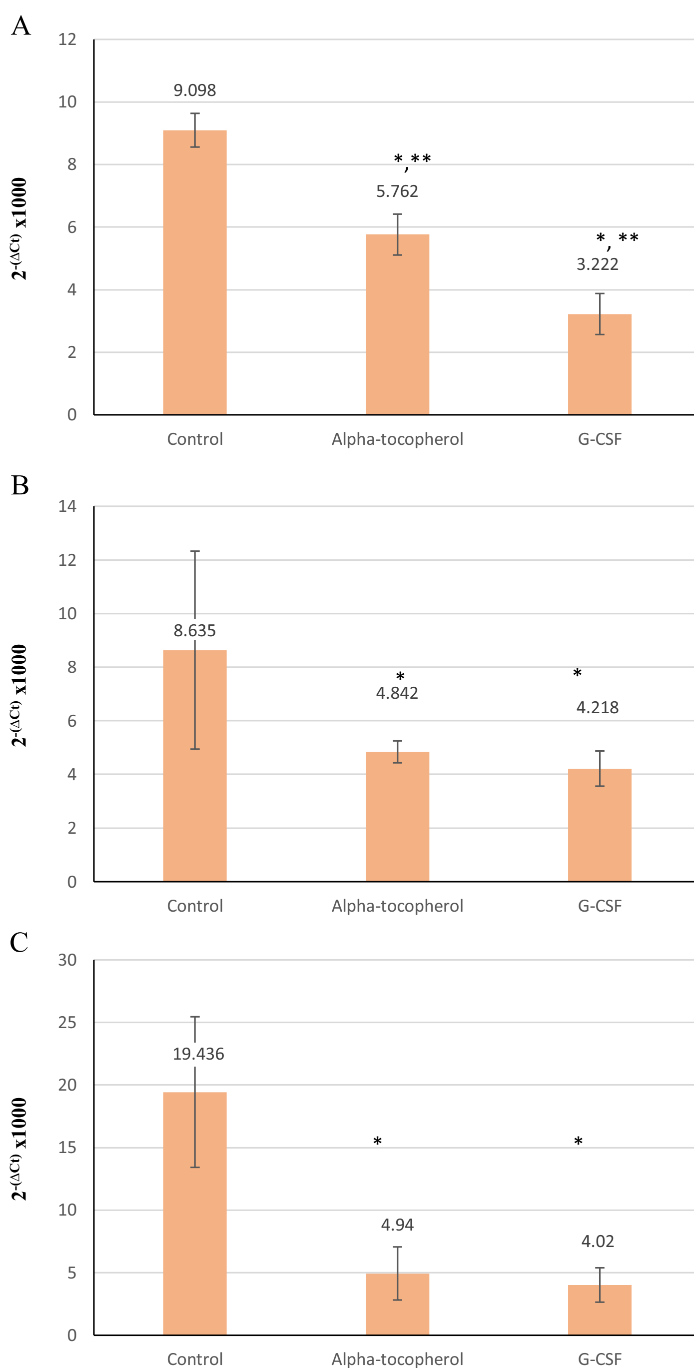

Figure 2.
The relative mRNA expression levels of SNAI1 (A), ALPP (B), *and* POUF51 (C) *genes. K562 cells were incubated either with alpha-tocopherol acetate at a dose of 100 µM (300 mg/mL stock solution; Lekchim, Ukraine) or G-CSF (Filstim, Biofarma, Ukraine) at a dose of 1 µg/mL (10,000 IU) for 72 h. Total RNA was extracted and converted to cDNA using Qiagen's QuantiTect Rev. Transcription Kit (Qiagen, Germany). A real-time RT-PCR was performed using the SYBR Green protocol. All the experiments were conducted in triplicate. The gene expression was quantified using the* $2^{-\Delta Ct}$ *method with normalization to GAPDH mRNA expression. ** $p < 0.01$ (A, C) *or* $p < 0.05$ (B) *compared to control cells; *** $p < 0.01$ *between the effects of alpha-tocopherol and G-CSF treatments.*

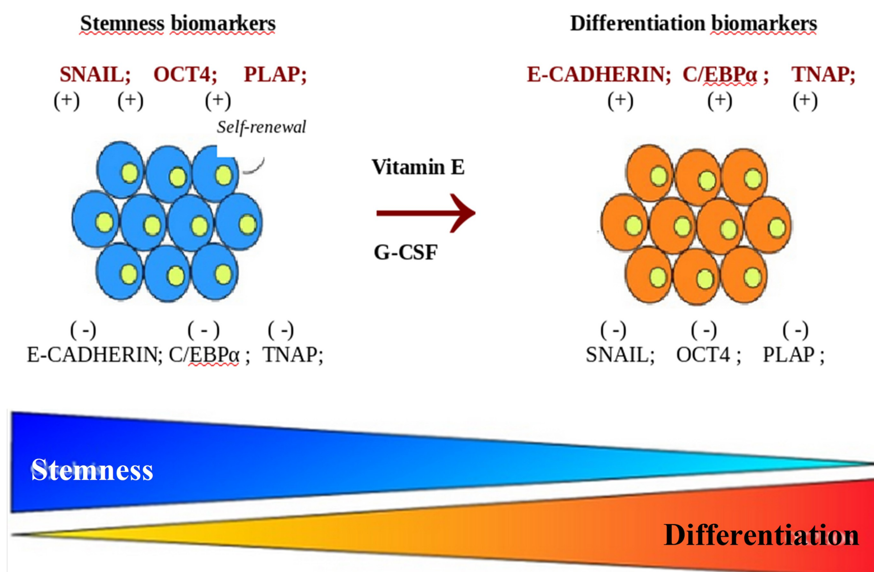

Figure 3.
Schematic diagram of the stimulated differentiation potential in CML blast cells by alpha-tocopherol and G-CSF. The repression of the stemness biomarkers SNAIL, OKT4, and PLAP results in the activation of the differentiation biomarkers E-cadherin, C/EBPα, and TNAP. Alpha-tocopherol decreases stemness markers expression with accompanying increase in differentiation potential. The diagram illustrates the reciprocal relations between stemness phenotype of stem-like blast cells and their differentiation potential with the balance between the transcription factors involved in cell differentiation and cell stemness.

Taken together, the results obtained have emphasized that the detected in vitro downregulation of the stemness biomarkers SNAIL, PLAP, and OKT4 at the RNA level in K562 CML cells, induced by alpha-tocopherol or G-CSF exposure, is accompanied by the activation of the expression of the differentiation biomarkers E-cadherin, C/EBPα, and TNAP. This suggests a complementary approach to CML therapy using agents that change the balance between the transcription factors involved in cell differentiation, on the one hand, and cell stemness, on the other hand, as part of differentiation therapy for myeloid leukemias. This is summarized schematically in **Figure 3,** illustrating the reciprocal relations between the leukemic pluripotent stemness phenotype and the differentiation potential of leukemic stem-like blast cells in CML myeloid differentiation, outlining the future strategies for differentiation therapy of CML.

3. USP1-targeted therapy as a novel strategy for chronic myeloid leukemia

While TKI therapy remains a mainstream approach in the treatment of CML in the chronic phase, the development of treatment resistance necessitates the search for novel strategies that involve numerous targets in cells other than BCR-ABL1 itself. Meanwhile, one of the major challenges in modern oncology is the pharmacological inaccessibility of many oncoproteins, primarily due to the absence of well-defined ligand-binding pockets or specific molecular targets.

Deubiquitinating enzymes (DUBs) provide an alternative strategy for the selective modulation of oncoprotein levels, as DUBs remove ubiquitin moieties from protein substrates, thereby regulating their stability, functional activity, and involvement in diverse cellular processes. DUBs play a pivotal role in maintaining cellular homeostasis by controlling a broad spectrum of essential functions, including the preservation of genomic integrity, regulation of gene expression, stem cell proliferation and differentiation, cell cycle progression, chromosome segregation during mitosis, and signal transduction pathways, particularly those mediated by growth factors. Alterations in their expression or enzymatic activity disrupt cellular equilibrium, ultimately contributing to the onset and progression of various pathological states, such as neurodegenerative disorders, immune system dysfunctions, and malignant neoplasms [32–34].

DUB inhibition may restore sensitivity to cytotoxic agents, a factor of particular importance for aggressive and recurrent cancer forms. Incorporating DUB inhibitors into therapeutic regimens holds the potential to enhance the efficacy of conventional chemo and radiotherapy. This synergistic interaction positions DUB-targeted strategies as a promising component of the next generation of anticancer therapies [35].

The role of several DUBs as putative targets for CML therapy has been demonstrated in various in vitro studies. In particular, DUBs are shown to be associated with the BCR-ABL1 signaling pathway, and the suppression of these enzymes may be an effective way to induce BCR-ABL1 ubiquitination and degradation [36, 37]. Thus, for example, the use of an inhibitor of USP9X results in BCR-ABL1 ubiquitination followed by the apoptosis of both TKI-sensitive and resistant CML cells [38]. Numerous data provide evidence that targeting DABs, in particular, USP7, USP9X, USP10, USP15, USP25, and USP47, can effectively overcome TKIs resistance in CML, which may represent a novel strategy for CML treatment [39].

Among approximately 100 DUBs, ubiquitin-specific protease 1 (USP1) stands out as one of the most extensively studied members. USP1 has emerged as a promising therapeutic target for various cancer types. Several inhibitors of USP1 slow down the growth of leukemic cell lines, including CML [40]. In our previous studies, we demonstrated that USP1 forms a protein complex with BCR-ABL1, a key driver of CML pathogenesis. Immunofluorescence analysis, followed by confocal microscopy, revealed that this protein complex localizes within the nuclei of CML cells [41]. The discovery of the USP1–BCR-ABL interaction prompted an investigation into the phosphorylation status of USP1. To this end, the phosphorylated USP1 isoforms were examined through immunoprecipitation of tyrosine-phosphorylated proteins, followed by immunofluorescence analysis in K562 cells, to assess their subcellular distribution. These experiments confirmed the presence of USP1 phosphorylated on tyrosine residues in CML cells [42]. Notably, the USP1 isoforms most prominently represented were those interacting with BCR-ABL1. Tyrosine-phosphorylated proteins were predominantly localized at the cell periphery, which likely reflects the high kinase activity of BCR-ABL1, as a substantial fraction of this oncoprotein also accumulates in these regions. Based on these findings, we hypothesize that BCR-ABL1 phosphorylates the deubiquitinase USP1, enhancing its catalytic activity. This post-translational modification may alter the balance of ubiquitination and deubiquitination processes, contributing to the disruption of cellular homeostasis and supporting leukemogenic signaling [8, 41, 42].

By deubiquitinating specific substrate proteins, USP1 plays a critical role in their stabilization, which is of particular interest for the development of novel therapeutic strategies in CML. In our study, we found that inhibition of USP1 deubiquitinating activity by a highly selective inhibitor, ML323 used in concentrations of 52 nM and 76 nM, resulted in a marked reduction of BCR-ABL1 levels in CML cells. A noticeable decrease in BCR-ABL1 levels was observed as early as 1.5 hours after ML323 administration, and the maximal effect was achieved in 3 hours. After 6 hours of exposure, BCR-ABL1 levels began to rise and returned to near-control levels by 12 hours (**Figure 4**). We hypothesize that this rebound may be attributed to either a loss of ML323 inhibitory activity or compensatory reactivation of USP1 via alternative mechanisms unaffected by the compound. Another plausible factor could be proteasomal overload, characterized by the impaired degradation of an abnormally large pool of misfolded proteins. This phenomenon, while extensively described in neurodegenerative diseases, remains insufficiently explored in cancer, despite the fact that malignantly transformed cells are characterized by elevated protein synthesis rates and significant accumulation of misfolded proteins [41, 43, 44].

The immunofluorescence analysis further revealed that ML323 treatment substantially reduced the colocalization of BCR-ABL and USP1, with the lowest levels detected at 1.5 h and 3 h of incubation, coinciding with the time points of minimal BCR-ABL1 abundance in cells [44].

Based on the results obtained, we believe that USP1 removes ubiquitin from the oncoprotein BCR-ABL1, thereby preventing its proteasomal degradation, which leads to its accumulation and subsequent disease progression. Therefore, USP1 could be considered a promising therapeutic target in CML. Lowering BCR-ABL levels through activation of cellular degradation systems represents a novel therapeutic approach for CML that does not depend on the mutational variability of ABL kinase domains and enables highly selective modulation of oncoprotein abundance, opening new avenues for USP1-targeted CML therapy.

It is also worth mentioning that, apart from the use of selective inhibitors of the DABs, one of the promising directions in developing targeted CML therapy involving the ubiquitin-proteasome system is represented by constructing bifunctional

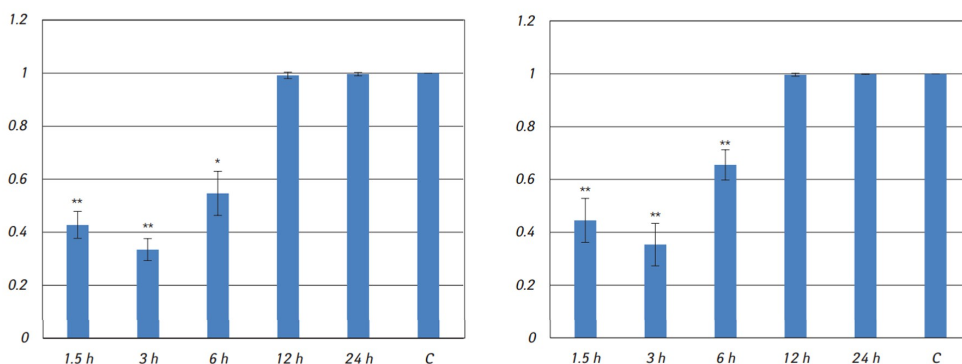

Figure 4.
Effects of ML323 — low-molecular-weight USP1 inhibitor — on BCR-ABL protein level after 24-h incubation with 52 nM (A) or 76 nM (B) ML323. The normalized values of band areas in Western blot of the lysates of CML K562 cells treated with ML323 are presented. Anti-BCR-ABL antibody (1:1000; MA1-153, Thermo Fisher Scientific, USA) was used for detection. The effect of ML323 was reversible, and in 12 h, the BCR-ABL level returned to the control values. * $p < 0.01$, ** $p < 0.001$ *as compared to controls.*

inhibitors that simultaneously target the BCR-ABL1 molecule and recruit E3 ubiquitin ligase [45]. Therefore, specific degradation of BCR-ABL1 may be achieved in several alternative ways.

4. Protein phosphatase 2A (PP2A) as an important node in kinase–phosphatase homeostasis and a presumable therapeutic target in CML

The prospective therapeutic modalities for CML targeted at BCR-ABL1 should consider various networks guiding the balance between the synthesis and degradation of this tyrosine kinase and the interactions of this oncoprotein with dozens of other molecules. One such molecule is protein phosphatase 2A (PP2A), which possesses tumor suppressor function [46]. This serine/threonine-specific phosphatase plays a key role in the regulation of multiple signaling pathways that control cell proliferation, differentiation, and apoptosis. PP2A exists as a heterotrimeric holoenzyme that includes three subunits: A (structural), B (regulatory), and C (catalytic) [47] (**Figure 5**)

PP2A functions primarily as a heterotrimer composed of a scaffold A (PPP2R1A/PPP2R1B), a catalytic C (PPP2CA/PPP2CB), and one of multiple regulatory B subunits (B/B55/PR55; B′/B56/PR61; B″/PR72/PPP2R3; B‴/striatin family/STRN). The combinatorial pairing of A/C with the numerous B-subunits yields more than 100 distinct holoenzymes, providing substrate specificity and directing PP2A to nuclear, cytoplasmic, or membrane-proximal locales [48]. The PP2A-A and PP2A-C exist in the form of a dimeric complex – core enzyme, while the regulatory B subunit exists as a single unit. The combination of the

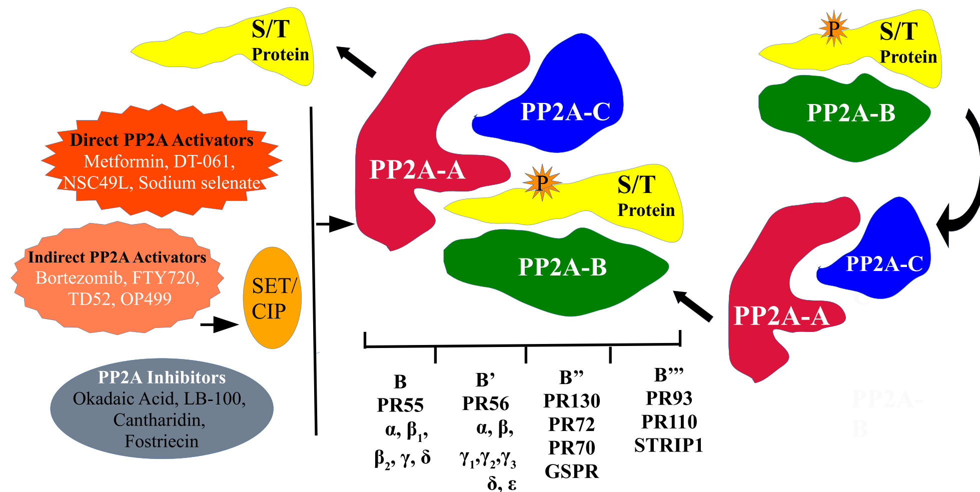

Figure 5.
Schematic representation of the formation of holoenzyme PP2A from subunits and modulators affecting the enzyme action. Each heterotrimeric PP2A holoenzyme consists of heterodimeric core enzyme, comprising a scaffolding subunit (A) and a catalytic subunit (C), and a variable regulatory subunit (B). Serine/threonine (S/T) phosphorylated (P) proteins interact with substrate-specific regulatory subunit (B), which subsequently recruits the substrate protein to the core enzyme. Holoenzyme formation is stabilized by the binding of the phosphorylated substrate to B subunit. The subunit C then dephosphorylates the substrate. The direct and indirect PP2A activators as well as PP2A inhibitors affecting the activity of PP2A are depicted.

dimer and trimer results in the formation of an active holoenzyme. PP2A thereby governs Wnt, mTOR, PI3K–AKT, and MAPK signaling, and regulates G1→S transition, DNA synthesis, and mitotic entry.

PP2A acts as a tumor suppressor, limiting the activity of oncogenic signaling cascades, including PI3K/AKT, MAPK, and JAK/STAT. PP2A inactivation is observed in many hematologic tumors and is associated with mutations, expression of endogenous inhibitors (e.g., SET), or interaction with oncoproteins [49]. In CML, BCR-ABL1 promotes PP2A inactivation via the activation of the SET inhibitor, which enhances oncogenic signaling [50]. It should be noted that A and C subunits are usually expressed unchanged in CML, and the suppression of PP2A activity is entirely determined by a significant decrease in B56α and partially in B55α subunits. B56α primarily affects the signaling pathways Wnt and p53, and B55α affects the pathways related to the mitotic cycle and metabolism (CDK1, Akt) [51, 52]. Detailing these interactions will further identify a specific subunit of PP2A complex as a possible therapeutic target. The restoration of PP2A activity leads to the suppression of clonal CML cells, including TKI-resistant ones.

Besides CML, in Ph-positive AML, the role of PP2A is also critical. Inactivation of the enzyme is associated with disruption of cell cycle control and increased proliferative potential. Experimental data indicate that activation of PP2A can lead to remission in preclinical models [53].

Therefore, the restoration of PP2A activity is considered a promising strategy for the treatment of CML and AML. The PP2A activators include FTY720 (fingolimod), OP449, DT-061, and small molecules that stabilize the holoenzyme [54, 55]. On the other hand, PP2A inhibitors, such as okadaic acid and cantharidin, are used in experimental studies to investigate the biological functions of the enzyme [56].

It is intriguing that PP2A in CML cells may be reactivated by targeting various molecules rather than PP2A itself. Among such targets, one can mention JAK [57]. The suppression of SET (PP2A inhibitor) in CML cells also brings about PP2A reactivation [58]. O'Connor et al. [59] stated that several different strategies aimed at PP2A reactivation may exist, namely inhibition of endogenous inhibitors of PP2A, targeted disruption of posttranslational modifications on PP2A subunits, and direct targeting of PP2A.

Combination approach to therapy seems to be the most optimal. Bringing the main first-line drug to the forefront, and based on this, possible practical recommendations for their use can be proposed. Foremost, priority should be given to identify the particular B-subunits expressed and involved in the disease setting (e.g., B56α vs. B56δ vs. B55α), since their substrate specificity and localization may differ substantially. Finally, the rational combination should be tailored. Because PP2A intersects with Wnt/mTOR/PI3K–AKT/MAPK, PP2A modulators may be combined with the appropriate kinase inhibitors or DNA-damaging agents, using biomarkers to avoid inactivation of tumor suppressor holoenzymes.

PP2A is at the center of cellular signaling homeostasis. Its heterotrimeric, B-subunit–directed organization explains both its reach and its tractability: by learning which holoenzymes are pathologically silenced (e.g., via SET or CIP2A) and which pathways they police, one can reactivate the right PP2A complexes or – in select contexts – transiently inhibit them to amplify therapeutic efficacy. This strategy is rapidly becoming actionable as better holoenzyme-selective chemical tools and robust methylation/B-subunit biomarkers emerge. Further research in this area is necessary to design clinically applicable drugs [59, 60].

5. Conclusion

While the introduction of TKIs into clinical practice turned out to be a revolution in CML treatment, being at the frontline of CML therapy for several decades, the acquired resistance to the therapy has become a growing source of concern. This brings to the forefront the development of novel treatment strategies and complementary therapies that may be helpful in overcoming treatment resistance, especially during the phase of the blast crisis. These novel approaches envisage the in-depth study of the BCR-ABL1 signaling pathways and the molecules interacting with this constitutively active tyrosine kinase. The differentiation therapy, proven as a valuable tool in the treatment of some types of cancer, may also be useful for CML treatment. It is of practical interest that several biologically active molecules, such as alpha-tocopherol, are as effective in vitro as G-CSF in upregulating the expression of factors associated with granulocyte differentiation. We demonstrated that the upregulation of the expression of the myeloid master regulator transcription factor C/EBPα, induced in CML cells by alpha-tocopherol or G-CSF, is accompanied by the downregulation of the stemness-related factors SNAIL, PLAP, and OKT4. The further search for applicable inducers of differentiation in CML cells should take into account that such substances may affect the balance between the transcription factors involved in cell differentiation and the factors involved in the maintenance of cell stemness.

Several methods that could be useful in CML therapy are focused not on the inhibition of the activity of the uncontrollably expressed BCR-ABL1 in CML cells but on the increased degradation of this tyrosine kinase, which finally leads to the decreased burden of the oncoprotein in the leukemic cells. Among such novel strategies are the methods targeting DUBs and PP2A since these enzymes are components of two major regulatory pathways determining the turnover of proteins and their activity. The challenging task in the practical development of these approaches is the search for the corresponding inhibitors possessing increased specificity, as well as the identification of the most appropriate components of the ubiquitin-proteasome system and the specific subunits of the PP2A system to serve as specific targets directed toward BCR-ABL1. Finally, combination therapy will open new prospects in CML treatment.

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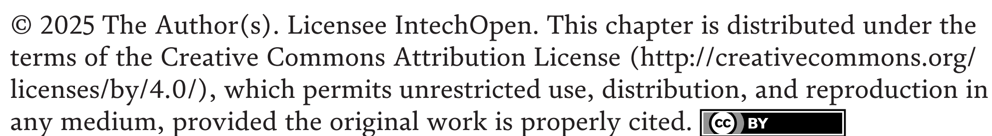

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